

PATENT SPECIFICATION

844,782

NO DRAWINGS.

Inventors:—HENRY BERNARD HAWLEY and RONALD HENRY HALL.



Date of filing Complete Specification : May 21, 1958.

Application Date : June 17, 1957. No. 19038/57.

Complete Specification Published : Aug. 17, 1960.

Index at Acceptance :—Class 2(3), AA(1B : 1C1C : 1C1D1 : 1C1D2 : 1C1E : 2B).

International Classification :—C12d.

COMPLETE SPECIFICATION.

The Production of Nisin.

We, APLIN & BARRETT LIMITED, a British Company, of Newton Road, Yeovil, Somerset, do hereby declare the invention, for which we pray that a patent may be granted 5 to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to the production of liquid or solid nisin preparations of high 10 nisin titre.

Nisin is the name given to the mixture of polypeptides produced by growing nisin-producing strains of *Streptococcus lactis* on an appropriate growth medium. The material is inhibitory of various micro organisms notably of many strains of *Clostridia*.

In accordance with the invention a concentrated nisin preparation is obtained from a dilute aqueous solution of nisin, produced 20 by growing in a culture medium a nisin-producing strain of *Streptococcus lactis* by a process in which a foam is produced from the aqueous solution in the presence of a small quantity, not exceeding 0.1% by 25 weight, of a surface active agent, and the foam is then collected. Conveniently the foam is collapsed prior to collection. The nisin may be precipitated from the recovered froth by salting out, and further purified by 30 redissolving it and re-precipitation.

The culture medium in which the *Streptococcus lactis* is grown in the first phase of the process may be a milk culture medium which comprises whole milk, skimmed milk 35 or reconstituted milk prepared from whole milk powder or skimmed milk powder. The conditions for growth of *Streptococcus lactis* in such medium for nisin production are already known. Generally, heat sterilisation 40 of the culture medium before inoculation is

desirable and it is also desirable that during the production of nisin the pH of the culture medium should be maintained at a value of about 6.0 by continuous or intermittent addition of alkali. When the production of 45 nisin is complete the pH of the culture medium may be brought to a value of about pH 5.8 to cause casein to separate as a curd or alternatively the caseinogen in the milk may be converted into paracasein by the addition of rennin or a similar enzyme.

The casein or paracasein is then separated 50 from the remaining culture medium or whey by physical methods such as skimming off the curd or draining off the whey, and the whey then transferred to a froth concentration plant after adding the surface active agent. A non-ionic surface active agent such as the product available under the Registered 55 Trade Mark "TWEEN 80" is suitable. Whilst the presence of the surface active agent is essential to enable an economic exhaustion of the nisin from the culture medium, the quantity specified above must not be exceeded since an excess tends to 60 displace nisin from the foam.

In the froth concentration plant upright 65 tubes are provided at the bottom with air distributors whereby air is introduced into the liquid which they contain and the whey is circulated through the lower ends of the tubes so as to form a short column at the lower ends.

The introduction of air at the bottom of 70 the tubes causes a foam to be produced and the foam reaching the top of the tubes contains the greater part of the nisin in the original whey. This foam is collected. It is preferable to collapse the foam before collection so as to reduce the volume to be collected 75 and this may be achieved by providing at the 80

top of each tube a rapidly rotating smooth disc.

The concentrate (i.e. collapsed foam or "spumate" as it is often called) which has

5 been collected may then be further worked up by salting out the nisin by the addition of sodium chloride, preferably acetone is also added to complete the collapsing of the foam. The solid precipitate obtained by salting out

10 may be re-dissolved in a solvent such as methanol and then again precipitated by the addition of a non solvent such as acetone.

The final product is a dry free flowing powder of high nisin titre.

15 The invention is further illustrated in the following example :—

A nisin producing strain of *Streptococcus lactis* is grown in a sterilized skim milk until

20 the nisin titre amounts to 1,000 Reading Units per ml. To 30 litres of this culture medium, at a pH within the range 6.0 to 6.3, rennet and calcium chloride are added to precipitate paracasein. The precipitated curd is cut up and the pH of the whey adjusted

25 to 4.5 by the addition of hydrochloric acid. The whey is drained off and the curd washed with water which has been acidified to a range 4.0 to 4.5 to remove nisin adhering to the curd and the washings and whey are

30 combined the pH being then adjusted to 5.0. This gives approximately 30 litres having a nisin titre of 800 Reading Units per ml.

The liquid is then transferred to a circu-

35 lating system having upright foam tubes and 0.1% of Tween 80 is added. Each tube is provided at the bottom with an air distributor so that foam rises up each tube and is collected at the top. In this way 550 ml. of

40 collapsed foam or spumate is collected having a nisin titre of 40,000 Reading Units ml. The collected spumate may be worked up to give a solid nisin product by saturating it with sodium chloride and adding 27 ml. of acetone. The resulting precipitate is removed and

45 extracted with 500 ml. of methanol, the solution then being centrifuged to remove any insoluble material. To the clear solution 1,000 ml. of acetone is added. The resulting active precipitate is dried in air or vacuo

50 and ground to give 11.9 grams of a white powder having a nisin titre of 1.4×10^6 Reading Units per gram.

It will be appreciated that though air is the most convenient gas for foam production,

any gas which is inert towards nisin may be used, such alternative gases being for example nitrous oxide, carbon dioxide or nitrogen.

WHAT WE CLAIM IS :—

1. A process for obtaining from a dilute aqueous solution of nisin, produced by growing in a culture medium a nisin producing strain of *Streptococcus lactis*, a concentrated nisin preparation, in which process a foam is produced from the aqueous solution in the presence of a small quantity not exceeding 0.1% by weight of a surface active agent, and the foam is then collected.

2. A process as claimed in Claim 1, in which the foam is collapsed prior to collection,

3. A process as claimed in Claim 1 or 2, in which the froth concentration is effected by transferring the culture medium to an upright tube having a rotating plate at the top and passing air upwardly through the tube, the foam collecting at the top of the tube being collapsed prior to collection by the rotating plate.

4. A process as claimed in any preceding claim, in which the nisin is recovered in solid form from the foam by salting out, re-dissolving the crude nisin in a solvent and precipitation of the final nisin preparation from the solution formed by addition of a non-solvent.

5. A process as claimed in Claim 4, in which sodium chloride is employed for salting out, methanol is employed as the solvent, and acetone is employed for precipitation from the solvent.

6. A process as claimed in any preceding claim, in which the dilute aqueous solution of nisin is formed by growing a nisin producing strain of *Streptococcus lactis* in a milk culture medium precipitating a curd of casein or paracasein from the culture medium, adjusting the pH of the culture medium to a value less than 4.5 and thereafter separating from the curd the aqueous liquid containing substantially all the nisin.

For the Applicants :
GILL, JENNINGS & EVERY,
Chartered Patent Agents,
51/52 Chancery Lane,
London, W.C.2.

PROVISIONAL SPECIFICATION.

The Production of Nisin.

We, APLIN & BARRETT, LIMITED, a British Company, of Newton Road, Yeovil, Somerset, do hereby declare this invention to be described in the following statement :—

105 The invention relates to the production of

liquid or solid nisin preparations of high nisin titre.

Nisin is the name given to the mixture of polypeptides produced by growing nisin-producing strains of *Streptococcus lactis* on

55

65

75

85

90

95

100

110

an appropriate growth medium. The material is inhibitory of various micro organisms notably of the strains of Clostridia responsible for much food spoilage.

5 In accordance with the invention nisin preparations are produced by a process comprising the steps of growing a nisin-producing strain of *Streptococcus lactis* in a culture medium containing milk caseinogen, precipitating casein or paracasein from the culture medium, bringing the *pH* to a value less than 4.5, separating the precipitated casein or paracasein and recovering the nisin in the whey by froth concentration.

10 The nisin may be precipitated from the recovered froth by salting out, and further purified by redissolving it and re-precipitation.

Nisin itself has surface active properties 20 but the addition of a small quantity, not exceeding 0.1% of an appropriate foaming agent will facilitate the production of froth. The culture medium in which the *Streptococcus lactis* is grown in the first phase of 25 the process may comprise whole milk, skimmed milk or reconstituted milk prepared from whole milk powder or skimmed milk powder. The conditions for growth of *Streptococcus lactis* in such medium for 30 nisin production are already known. Generally, heat sterilisation of the culture medium before inoculation is desirable and it is also desirable that during the production of nisin the *pH* of the culture medium should be 35 maintained at a value of about 6.0 by continuous or intermittent addition of alkali. When the production of nisin is complete the *pH* of the culture medium may be brought to a value of about *pH* 5.8 to cause casein to 40 separate as a curd or alternatively the caseinogen in the milk may be converted into paracasein by the addition of rennin or a similar enzyme.

The casein or paracasein is then separated 45 from the remaining culture medium or whey by physical methods such as skimming off the curd or draining off the whey, and the whey then transferred to a froth concentration plant.

50 In this plant upright tubes are provided at the bottom with air distributors whereby air is introduced into the liquid which they contain and the whey is circulated through the lower ends of the tubes so as to form a 55 short column at the lower ends. The addition of 0.1% of a non-ionic surface active agent such as a partial higher fatty acid ester of a polyhydroxy alcohol (e.g. the substance available under the Registered Trade Mark 60 "TWEEN 80") is advantageous.

The introduction of air at the bottom of the tubes causes a foam to be produced and the foam reaching the top of the tubes contains the greater part of the nisin in the original 65 whey. This foam is collected. It is preferable

to collapse the foam before collection so as to reduce the volume to be collected and this may be achieved by providing at the top of each tube a rapidly rotating smooth disc.

The concentrate (i.e. collapsed foam or "spumate" as it is often called) which has been collected may then be further worked up by salting out the nisin by the addition of sodium chloride, preferably acetone is also added to complete the collapsing of the foam. The solid precipitate obtained by salting out 70 may be re-dissolved in methanol and then again precipitated by the addition of acetone. 75

The final product is a dry free flowing powder of high nisin titre.

The invention is further illustrated in the following example.

A nisin producing strain of *Streptococcus lactis* is grown in a milk culture until the nisin titre amounts to 1,000 Reading Units 80 per ml. To 30 litres of this culture medium, at a *pH* within the range 6.0 to 6.3, rennet and calcium chloride are added to precipitate paracasein. The precipitated curd is cut up and the *pH* of the whey adjusted to 4.5 by the addition of hydrochloric acid. The whey is drained off and the curd washed with water which has been acidified to a range 4.0 to 4.5 to remove nisin adhering to the curd and the washings and whey are combined the *pH* being then adjusted to 5.0. This gives approximately 30 litres having a nisin titre 90 of 800 reading units per ml.

The liquid is then transferred to a circulating system having upright foam tubes and 100 0.1% of Tween 80 is added. Each tube is provided at the bottom with an air distributor so that foam rises up each tube and is collected at the top. In this way 550 ml. of collapsed foam or spumate is collected having a nisin titre of 40,000 Reading Units per ml. The collected spumate may be worked up to 105 give a solid nisin product by saturating it with sodium chloride and adding 27 ml. of acetone. The resulting precipitate is removed and extracted with 500 ml. of methanol, the solution then being centrifuged to remove any insoluble material. To the clear solution 1,000 ml. of acetone is added. The resulting active precipitate is dried in air or vacuo and 110 ground to give 11.9 grams of a white powder having a nisin titre of 1.4×10^6 Reading Units per gram.

It will be appreciated that though air is the 115 most convenient gas for foam production, any gas which is inert towards nisin may be used, such alternative gases being for example nitrous oxide, carbon dioxide or nitrogen.

For the Applicants :—

GILL, JENNINGS & EVERY,
Chartered Patent Agents,
51/52 Chancery Lane,
London, W.C.2.